

Fig. S1. *TgBAC(pdgfrb:gal4ff)^{ncv24}; Tg(UAS:GFP)^{nksuasgfp1a}; Tg(-0.8flt1:RFP)^{hu5333}* transgenic fish mark mural cells on fin blood vessels. Maximum intensity projections of confocal z-stacks in lateral views with anterior to the left. **(A)** Caudal fin of 4 weeks post fertilization (wpf) fish. Scale bar: 100 μ m. S1-S4 represent the four segments used for quantifying the distribution of GFP expressing cells. **(B)** Proximal segment of caudal fin blood vessel. Scale bar: 10 μ m. Boxed region enlarged in **(B')** shows GFP cells wrapping around blood vessel. Scale bar: 7 μ m. **(C-E)** Mid-vessel and distal segments of caudal fin blood vessel. Scale bar: 10 μ m. Boxed regions enlarged in **(D',E')** show GFP positive cells with protrusions (red arrowheads). Scale bar: 7 μ m. Quantification of images can be found in **Figure 1K**.

TgBAC(myh11a:YFP)^{mu125}; Tg(-0.8flt1:RFP)^{hu5333}

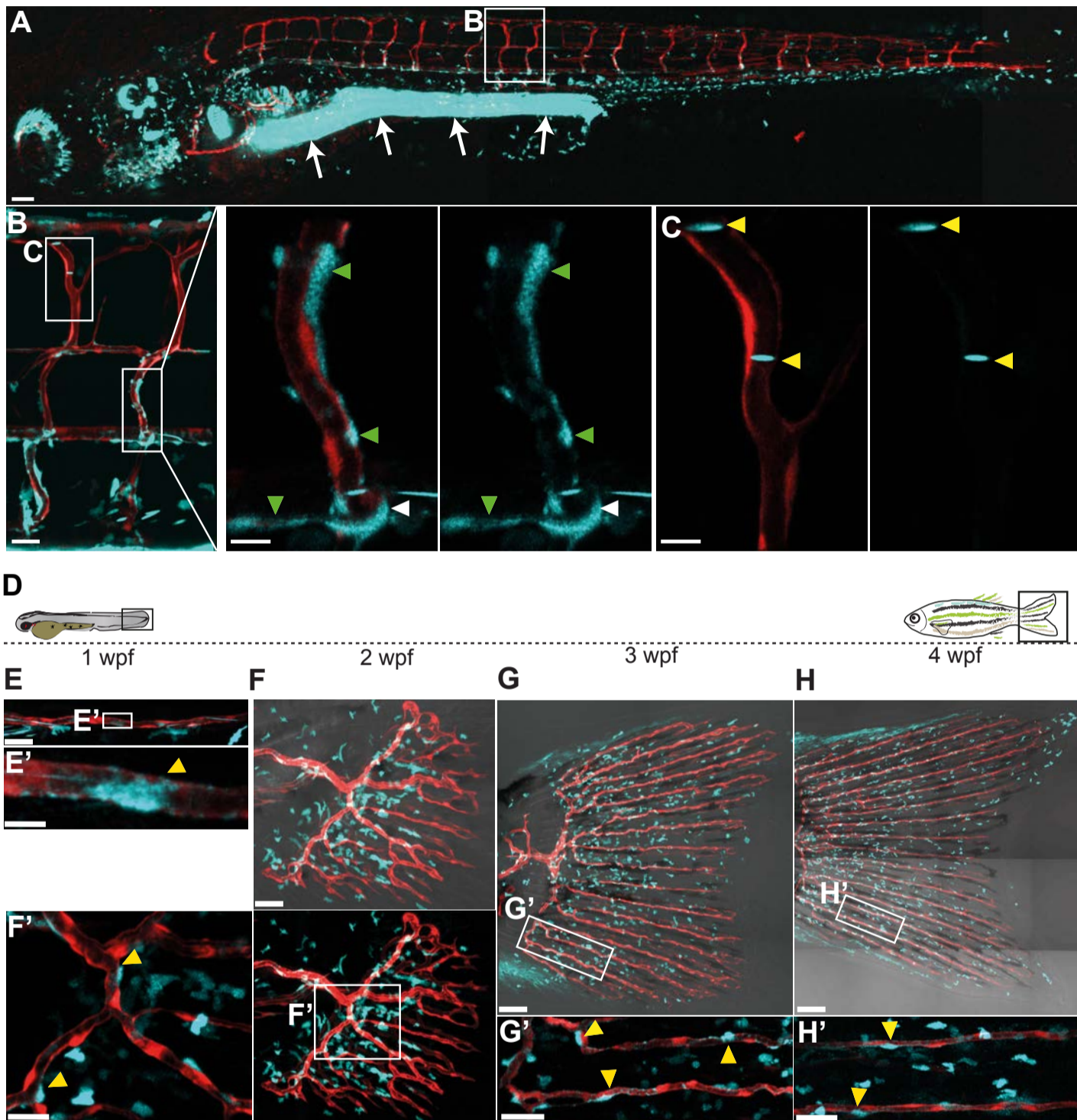
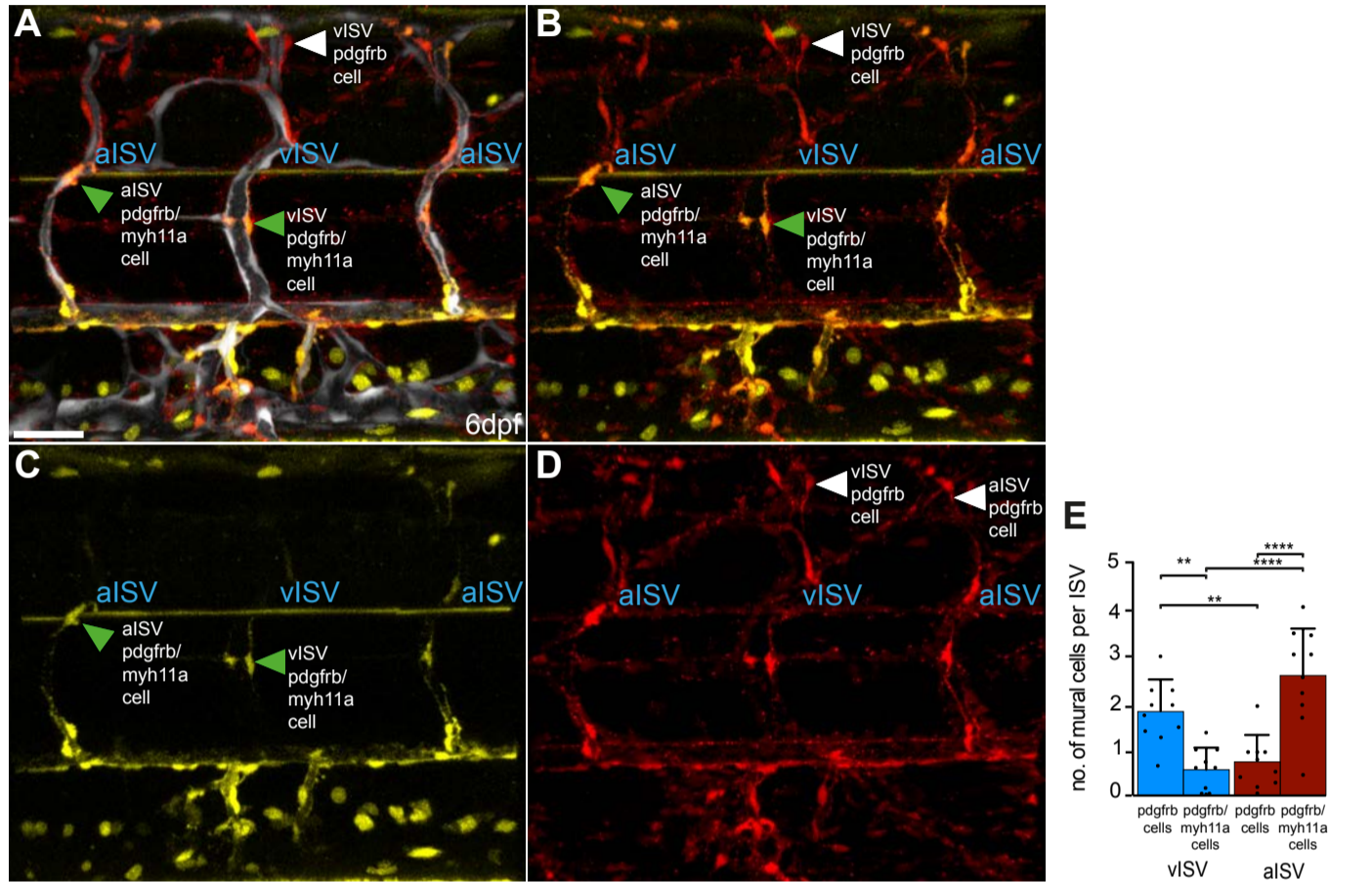


Fig. S2. *TgBAC(myh11a:YFP)^{mu125}* marks mural cells of trunk and caudal fin vessels.

Maximum intensity projections of confocal z-stacks of *Tg(-0.8flt1:RFP)^{hu5333}*, *TgBAC(myh11a:YFP)^{mu125}* double transgenic fish. **(A)** 5 days post fertilization (dpf) fish showing arterial ECs (red) and YFP positive cells (blue). Arrows mark expression in gut smooth muscle cells. Scalebar: 200 μm **(B)** Intersegmental vessels (ISVs). Scale bar: 20 μm . Enlarged boxed area shows YFP cells next to arterial ISVs (green arrowheads) and on the ISV entrance from the dorsal aorta (white arrowhead). Scale bar: 5 μm . **(C)** Expression of YFP in cells in circulation (yellow arrowheads). Scale bar: 5 μm . **(D)** Expression of YFP during juvenile fin development. Schematic representation of the time course of the study. **(E)** YFP positive cells colonize the caudal fin sprout by 1 week post fertilization (wpf). Scale bar: 30 μm . Boxed area enlarged in **(E')** shows the presence of YFP cells on arteries (yellow arrowhead). Scale bar: 8 μm . **(F)** Developing vasculature at 2 wpf. Scale bar: 30 μm . Boxed area enlarged in **(F')** shows YFP positive cells colonizing blood vessels (yellow arrowheads). Scale bar: 8 μm . **(G)** Vasculature at 3 wpf. Scale bar: 50 μm . Boxed region enlarged in **(G')** shows YFP in association with blood vessels (yellow arrowheads). Scale bar: 10 μm . **(H)** Caudal fin vasculature at 4 wpf. Scale bar: 100 μm . Boxed region enlarged in **(H')** shows YFP cells on arteries (yellow arrowheads). Scale bar: 10 μm .

TgBAC(pdgfrb:mCherry)^{ncv23}; TgBAC(myh11a:YFP)^{mu125}; Tg(kdrl:TagBFP)^{mu293}



F *TgBAC(acta2:mCherry)^{ca8}; Tg(myh11a:YFP)^{mu125}; Tg(kdrl:TagBFP)^{mu293}*

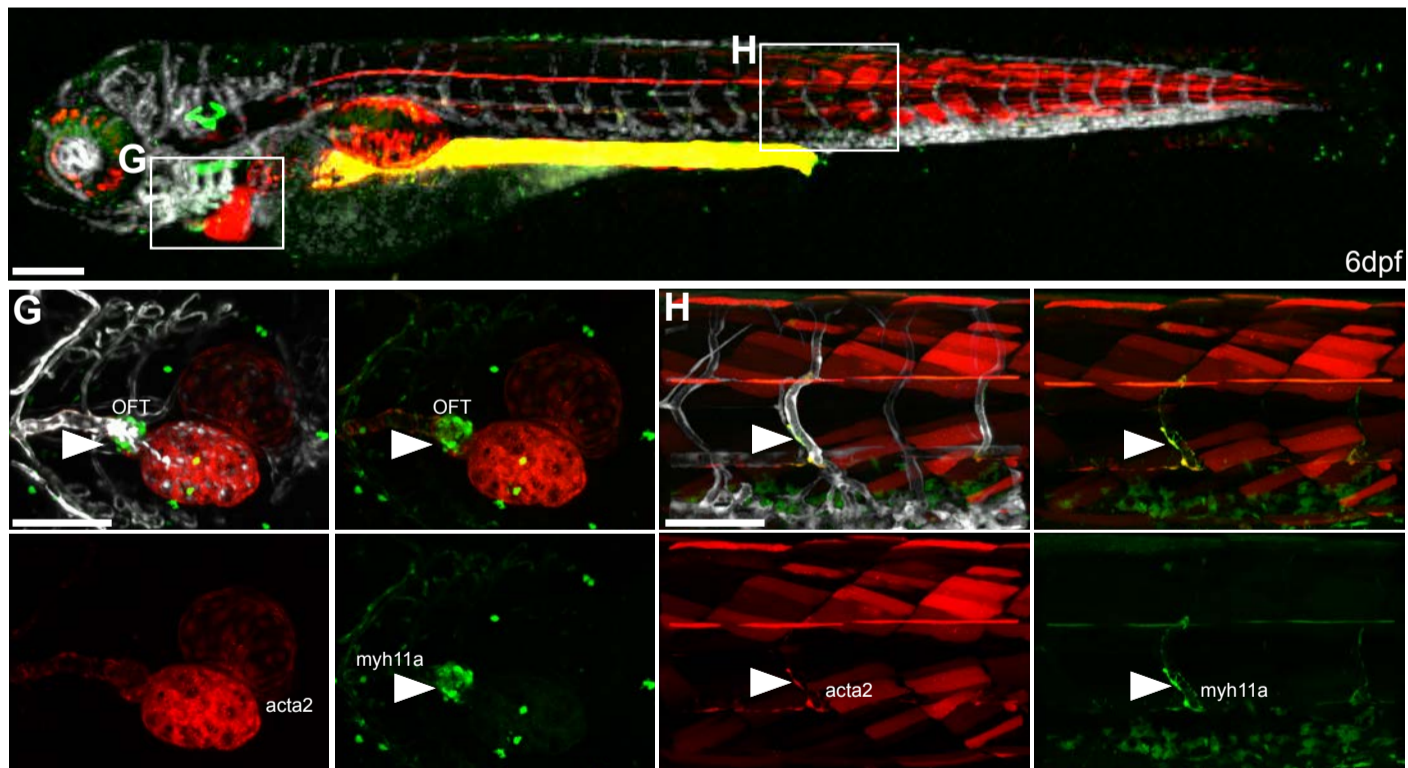


Fig. S3. Colocalization of myh11a and pdgfrb in cells of embryonic trunk blood vessels.

(A-D) Maximum intensity projections of confocal z-stacks of *Tg* *BAC(pdgfrb:mCherry)^{ncv23}; TgBAC(myh11a:YFP)^{mu125}; Tg(kdrl:TagBFP)^{mu293}* triple transgenic fish labelling all ECs (blue), pdgfrb positive cells (red) and myh11a positive cells (yellow). Lateral views, anterior to the left. Trunk ISVs of 6 dpf zebrafish larvae show pdgfrb (white arrowheads) and pdgfrb/myh11a double-positive (green arrowheads) cells associating with the vasculature. Scale bar: 20 μ m. (E) Distribution of pdgfrb and pdgfrb/myh11a double-positive cells per ISV. Note an increase of double positive cells on arterial intersegmental vessels (aISVs), while pdgfrb only positive cells were enriched on the venous intersegmental vessels. One-way ANOVA n=11. n.s., not significant, **P=0018, ****P= <0.0001, Data are mean \pm s.d. Data points indicate individual larvae. (F,H) Maximum intensity projections of confocal z-stacks of *TgBAC(acta2:mCherry)^{ca8}; TgBAC(myh11a:YFP)^{mu125}; Tg(kdrl:TagBFP)^{mu293}* fins, labelling acta2 (red), myh11a (yellow), endothelial cells (white). Larvae of 6 dpf zebrafish. Scale Bar: 400 μ m. (G) acta2 expression was observed in atrium, ventricle, and out-flow tract, myh11a expression was restricted to out-flow tract (white arrowheads). Scale bar: 20 μ m. (H) acta2 expressing mural cells of trunk intersegmental vessels also express myh11a (white arrowheads). Scale bar: 20 μ m.

TgBAC(pdgfrb:gal4ff)^{ncv24}; Tg(UAS:GFP)^{nkuasgfp1a}; Tg(TP1bglobin:H2B-mCherry)^{s939}

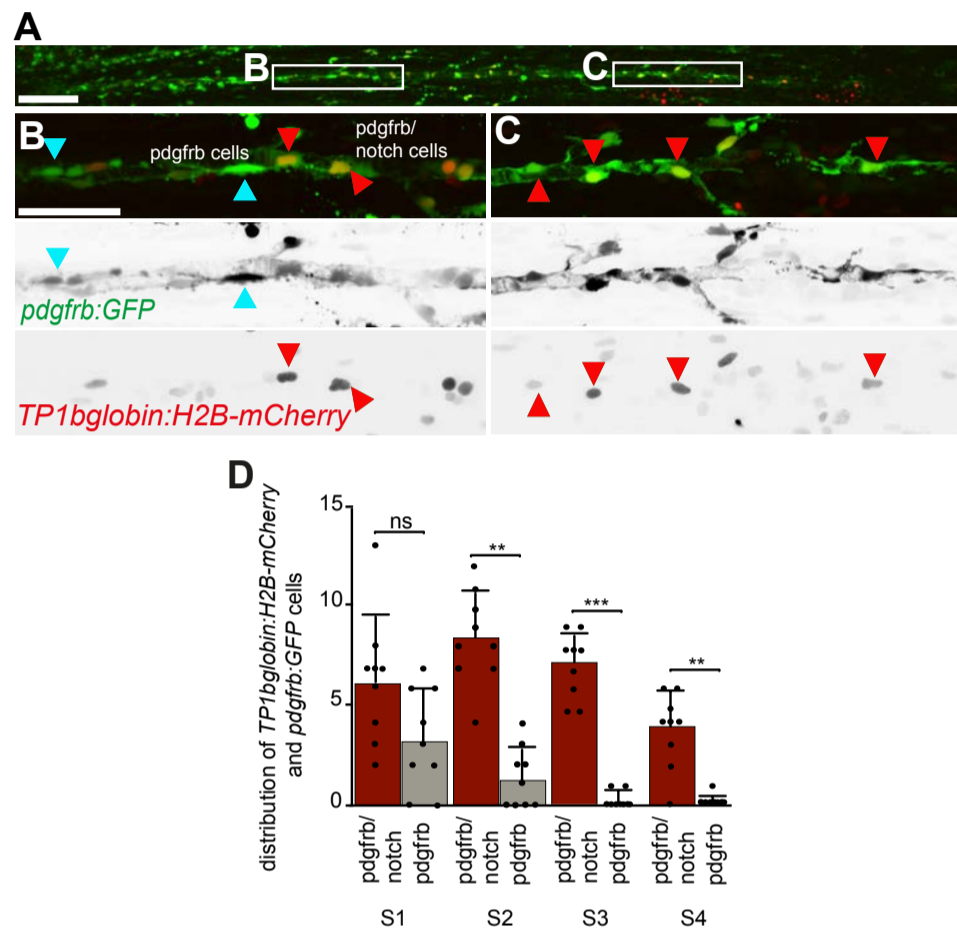


Fig. S4. Mural cells along the proximal distal axis display differential notch activity.

(A-C) Maximum intensity projections of confocal z-stacks of *TgBAC(pdgfrb:gal4ff)^{ncv24}; Tg(UAS:GFP)^{nkuasgfp1a}; Tg(TP1bglobin:H2B-mCherry)^{s939}* fins, labelling pdgfrb positive cells (green) and mCherry positive cell nuclei (red). (A, B) Caudal fin of 4 wpf fish. Scale bar: 70 μ m for (A), 10 μ m for (B). (B) Mural cells in the proximal segments express only pdgfrb (blue arrowheads) or pdgfrb/notch (red arrowheads) (C) Mural cells in the distal region express pdgfrb and have activated notch signalling (red arrowheads). (D) Distribution of pdgfrb and pdgfrb/notch positive mural cells. Note an increase in mural cells expressing pdgfrb only in proximal segments. One-way ANOVA. n=9 rays from four individual fish (average length: 1676 μ m) n.s., not significant, **P= 0.0017, 0.0041, ****P=<0.0001, Data are mean \pm s.d.

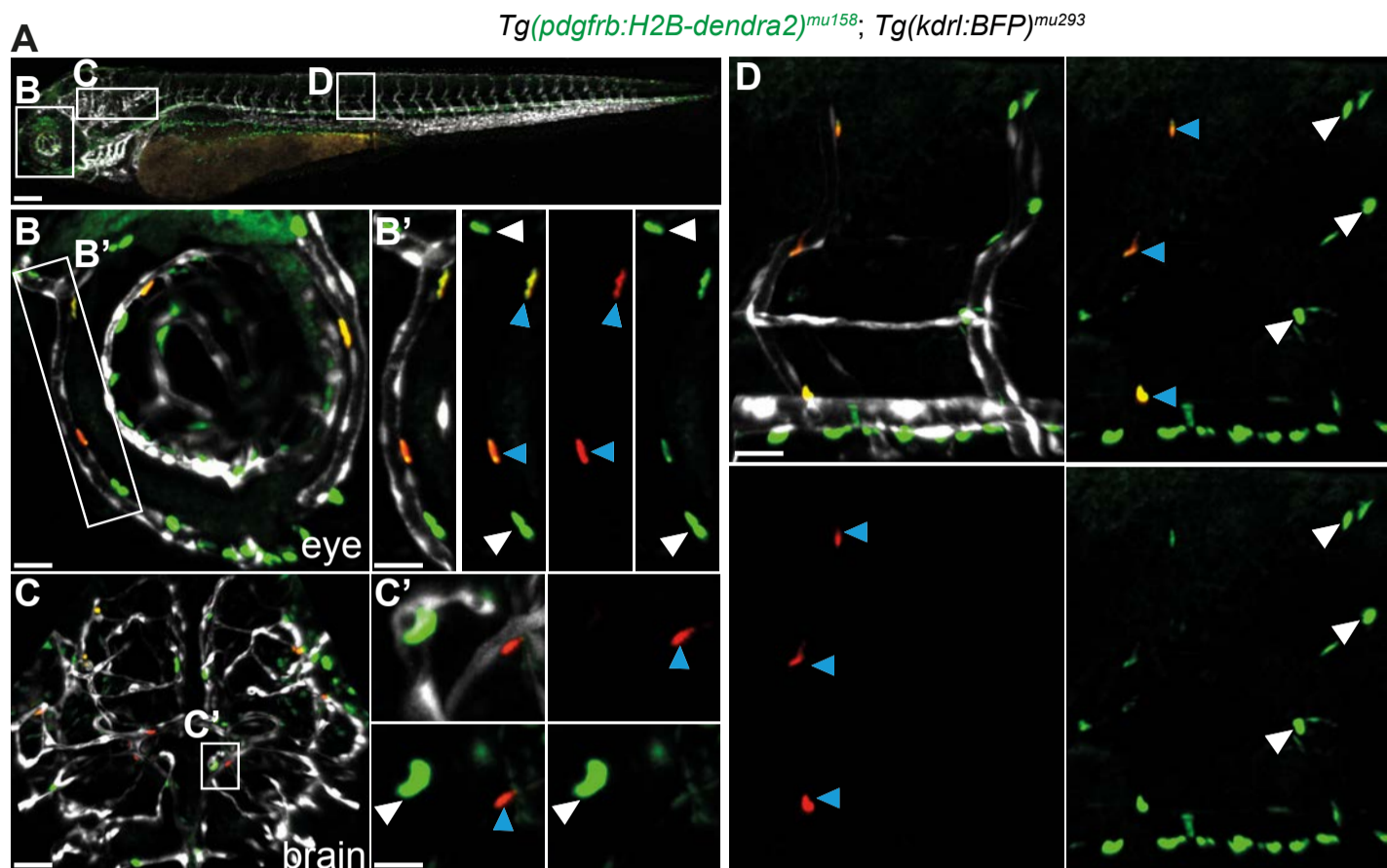


Fig. S5. *Tg(pdgfrb:H2B dendra2)^{mu158}* labels cell nuclei of *pdgfrb* expressing cells.

Maximum intensity projections of confocal z-stacks of *TgBAC(pdgfrb:H2B-dendra2)^{mu158}; Tg(kdrl:BFP)^{mu293}* double transgenic fish labelling the nuclei of *pdgfrb* positive cells with the photoconvertible protein Dendra2 (green) and all endothelial cells (white). **(A)** 3 dpf embryo showing *pdgfrb* cells expressing Dendra2 protein in different vascular beds. Scale bar: 200 μ m **(B)** Presence of Dendra2 positive cells along eye blood vessels. Scale bar: 20 μ m. Boxed area enlarged in **(B')** shows the inner optic circle with photoconverted cells (blue arrowheads) and unconverted cells (white arrowheads). Scale bar: 10 μ m. **(C)** Head vessels of 3 dpf zebrafish, anterior to top. Scale bar: 20 μ m. Boxed regions enlarged in **(C')** shows un-converted (white arrowheads) and photoconverted (blue arrowheads) cells on the central arteries. Scale bar: 10 μ m. **(D)** Inter-segmental vessels and dorsal aorta show un-converted (white arrowheads) and photoconverted (blue arrowheads) Dendra2 expressing cells. Scale bar: 15 μ m.

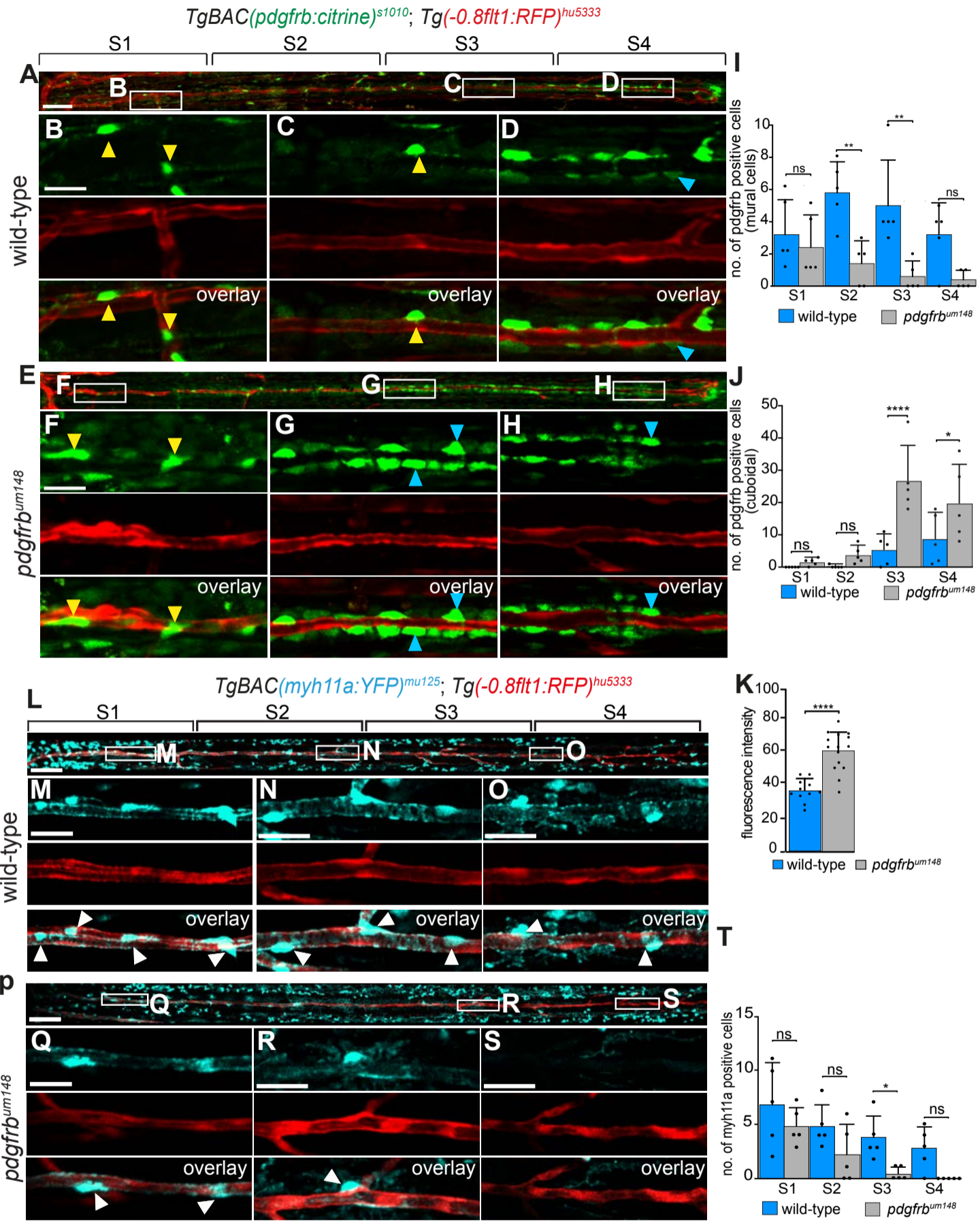


Fig. S6. Mural cell recruitment to caudal fin arteries requires *pdgfrb* signalling. Maximum intensity projections of confocal z-stacks of *Tg(-0.8flt1:RFP)^{hu5333}*, *TgBAC(pdgfrb:citrine)^{s1010}* fish. **(A)** Caudal fin artery in wildtype fish at 5 wpf. Scale bar: 70 μm . **(B, C)** Proximal and mid-vessel segments showing citrine expressing mural cells (yellow arrowheads). Scale bar: 10 μm . **(D)** Distal segment of caudal fin containing citrine expressing cuboidal cells (blue arrowhead). **(E)** Caudal fin vessel of *pdgfrb^{um148}* mutant fish at 5 wpf. Scale bar: 50 μm . **(F, G)** Proximal and mid-vessel segments contain citrine expressing mural cells (yellow arrowheads) and cuboidal cells (blue arrowheads). Scale bar: 10 μm . **(H)** Distal segment with cuboidal cells (blue arrowhead). **(I)** Quantification of citrine expressing mural cell distribution across fin segments of wild-type and *pdgfrb^{um148}* fish. One-way ANOVA. n.s., not significant, **P= 0.0024, Data are mean \pm s.d. **(J)** Quantification of citrine expressing cuboidal cells in wild-type and *pdgfrb^{um148}* mutant fish. One-way ANOVA. n=5 fish per group. n.s., not significant, Data are mean \pm s.d. *P<0.0494, ****P=0.0001. **(K)** Fluorescent intensity of cuboidal cells in wild-type and *pdgfrb^{um148}* fish. Un-paired t-test ****P=0.0001. Data are mean \pm s.d. **(L)** Maximum intensity projections of confocal z-stacks of *Tg(-0.8flt1:RFP)^{hu5333}*, *TgBAC(myh11a:YFP)^{mu125}* double transgenic fish. Scale bar: 80 μm . **(M, N)** Proximal and mid-vessel segments (boxed regions in **(M)**) shows YFP positive mural cells (white arrowheads). Scale bar: 15 μm **(M)**, 10 μm **(N)**. **(O)** YFP positive cells (white arrowheads) in distal segments. Scale bar: 10 μm **(P)** Caudal fin vessel of *pdgfrb^{um148}* mutants. Scale bar: 80 μm . **(Q, R)** Proximal and mid-vessel segments showing YFP positive mural cells (white arrowheads). Scale bar: 15 μm **(P)**, 10 μm **(Q)**. **(S)** Distal segment of caudal fin lacking YFP positive cells. Scale bar: 10 μm . **(T)** Quantification of YFP positive cells in wildtype and *pdgfrb^{um148}* mutant fish. One-way ANOVA. n=5 fish per group, n.s., not significant, *P=0.0422. Data are mean \pm s.d.

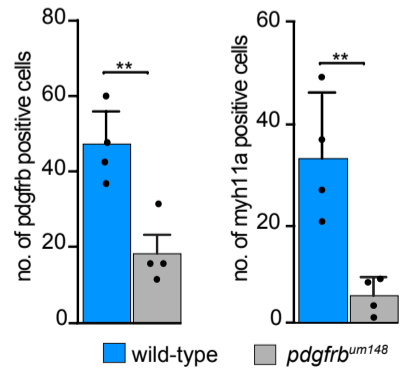
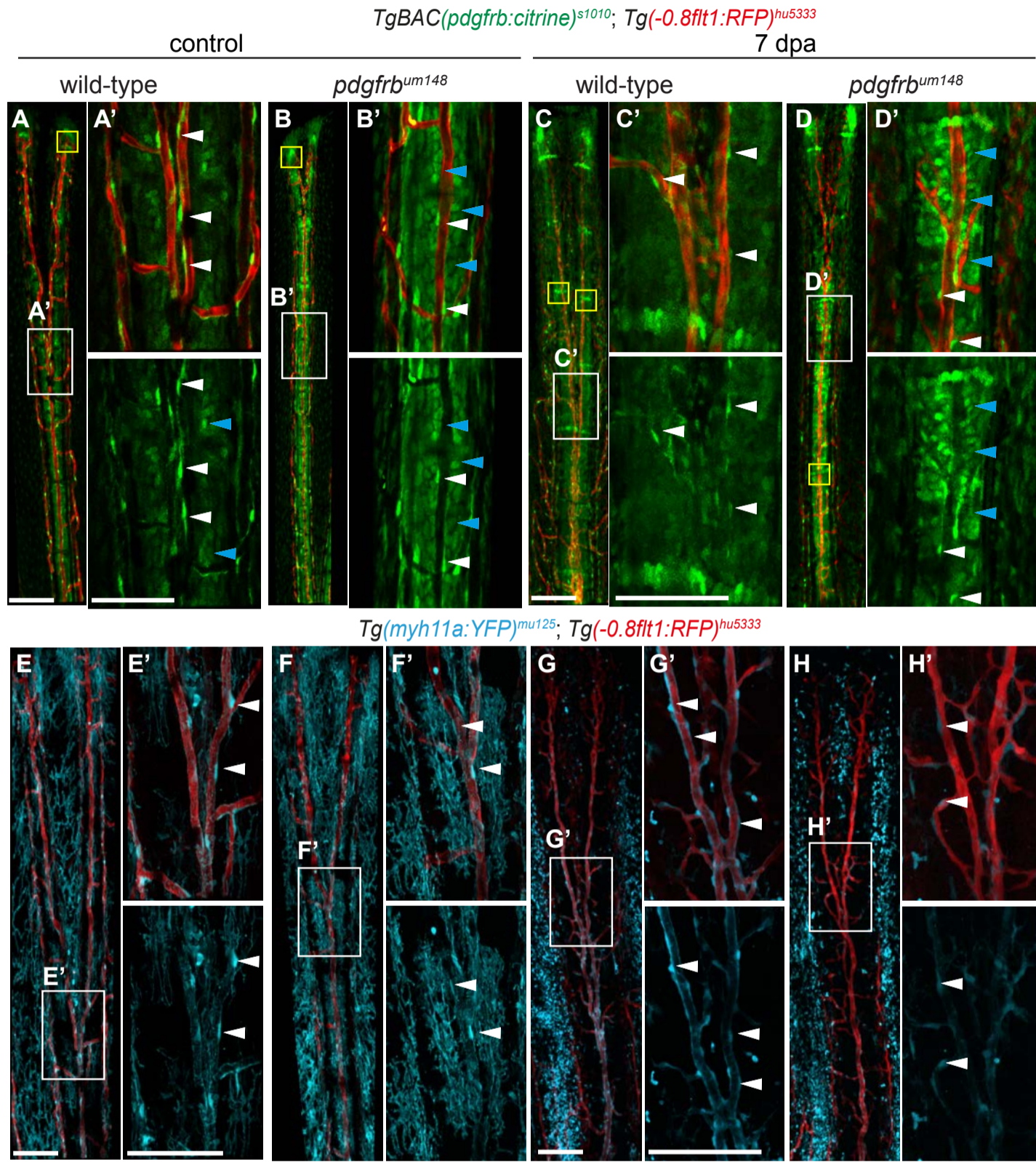


Fig. S7. Mural cell recruitment to un-amputated and regenerated fin rays of the caudal fin.

Maximum intensity projections of confocal z-stacks of *TgBAC(pdgfrb:citrine)^{S1010}; Tg(-0.8flt1:RFP)^{hu5333}* double transgenic fish labelling arterial ECs (red) and *pdgfrb* positive cells (green). **(A, B)** Caudal fin blood vessels of wild-type and *pdgfrb^{um148}* mutant fish. Scale bar: 50 μ m. Note the presence of oval-shaped cells in terminal regions of the fin ray (yellow box). Boxed regions enlarged in **(A'** and **B')** show presence of mural cells along blood vessels (white arrowheads). Cuboidal cell numbers are increased in mutants (blue arrowheads). Scale bar: 40 μ m. **(C, D)** Regenerated caudal fin blood vessels of wild-type and *pdgfrb^{um148}* mutants at 7 days post amputation (dpa). Scale bar: 50 μ m. Boxed regions enlarged in **(C'** and **D')** show presence of mural cells along regenerated blood vessels (white arrowheads). Scale bar: 40 μ m. Note increase of cuboidal cells in mutants (blue arrowheads). Maximum intensity projections of confocal z-stacks of *Tg(-0.8flt1:RFP)^{hu5333}; TgBAC(myh11a:YFP)^{mu125}* double transgenic fish labelling arterial ECs (red) and *myh11a* positive cells (blue). **(E, F)** Caudal fin blood vessels of wild-type and *pdgfrb^{um148}* mutants. Scale bar: 150 μ m. Boxed regions enlarged in **(E'** and **F')** show the presence of mural cells along blood vessels (white arrowheads). Scale bar: 40 μ m. **(G, H)** Regenerated caudal fin blood vessels of wild-type and *pdgfrb^{um148}* mutants at 7 dpa. Scale bar: 500 μ m. Boxed regions enlarged in **(G'** and **H')** show mural cells (white arrowheads). Scale bar: 50 μ m. **(I)** Quantification of *pdgfrb* and *myh11a* positive cells recruited to regenerated blood vessels. **P<0.005.

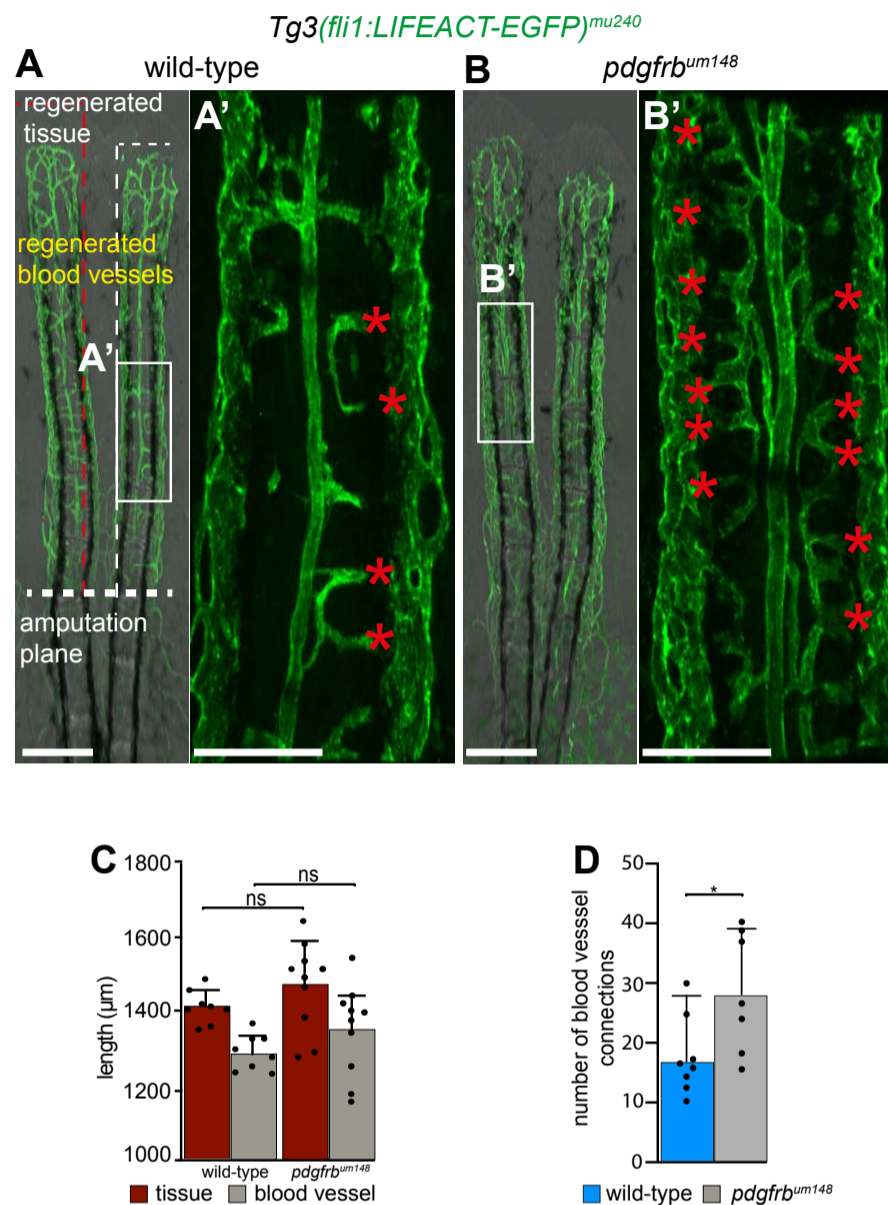


Fig. S8. Loss of *pdgfrb* signalling does not impair blood vessel or tissue regeneration.

Maximum intensity projections of confocal z-stacks of *Tg3(fli1: LIFEACTION-EGFP)^{mu240}* labelling endothelial cells (green). (A) wild-type and (B) *pdgfrb^{um148}* mutants, dashed lines represent length of regenerated tissue (red dotted lines) and blood vessel (white dotted lines). Boxed regions enlarged in (A') and (B') show blood vessel connections (red asterisks). (C) Quantification for regenerated tissue and blood vessel length. Regeneration length of tissue or blood vessel is not impaired in mutants. One-way ANOVA. n.s., not significant. wildtype n=4, mutant n=5. Data points represent individual fin rays. Data are mean±s.d. (D) Quantification for blood vessel connections in wildtype and mutants. Note the increase in connections in the mutants. Un-paired t-test *P=0.0258, n=4 fish. Data points represent individual fin rays. Data are mean±s.d.

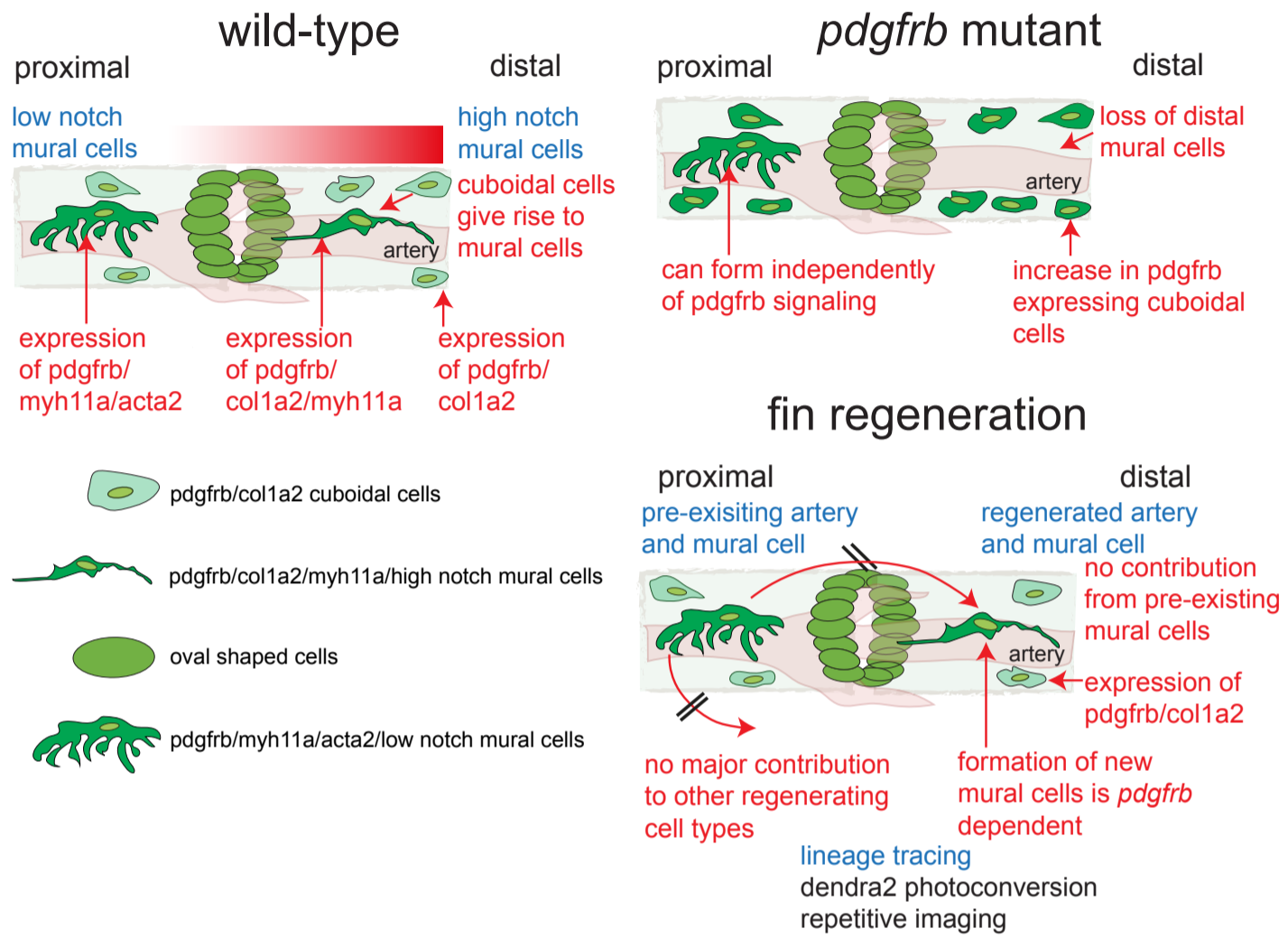
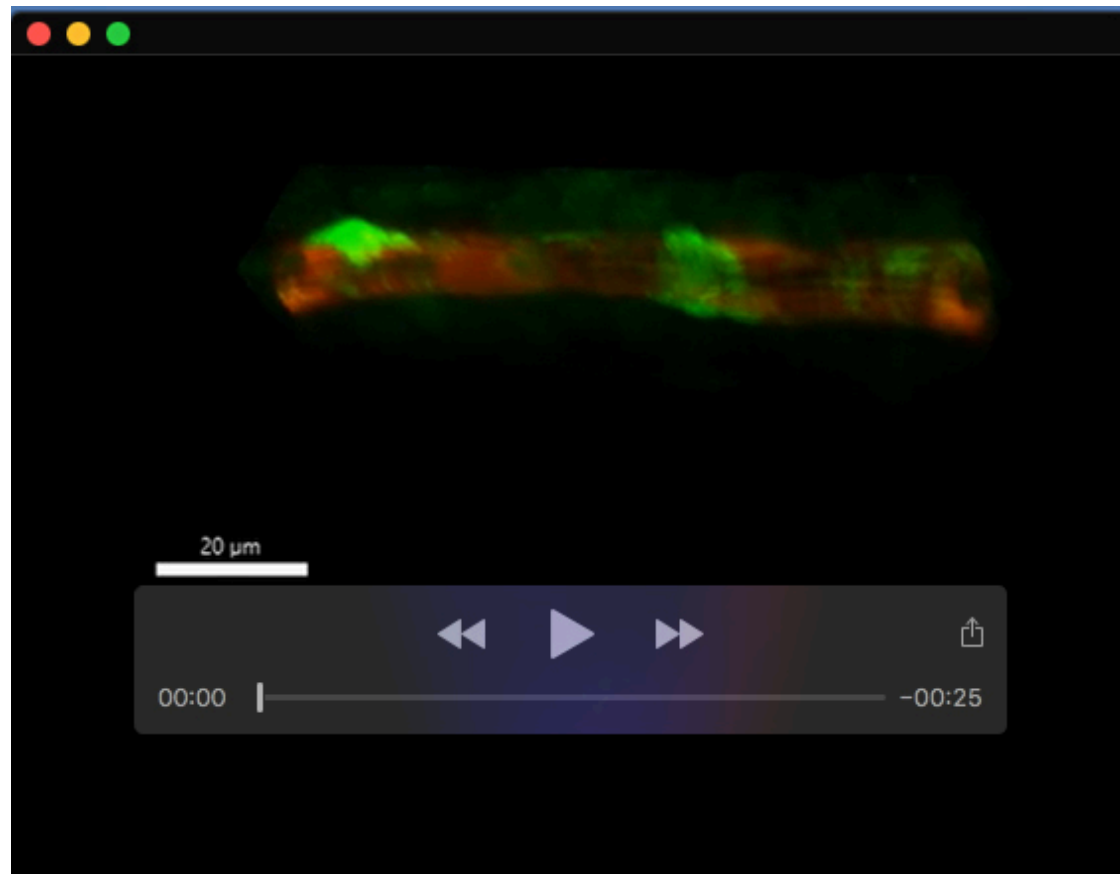


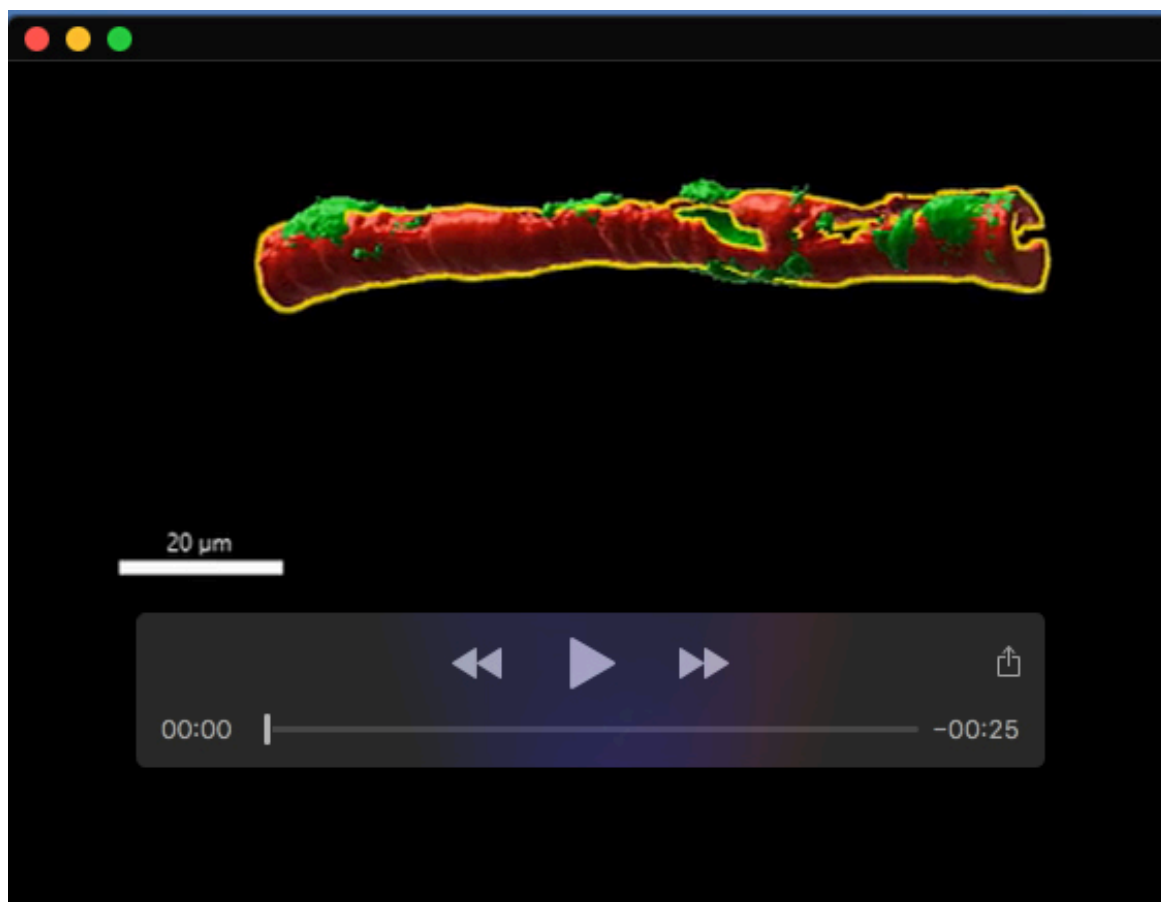
Fig. S9. Schematic representation of our findings.

Table S1. Primer sequences for qPCR experiments.

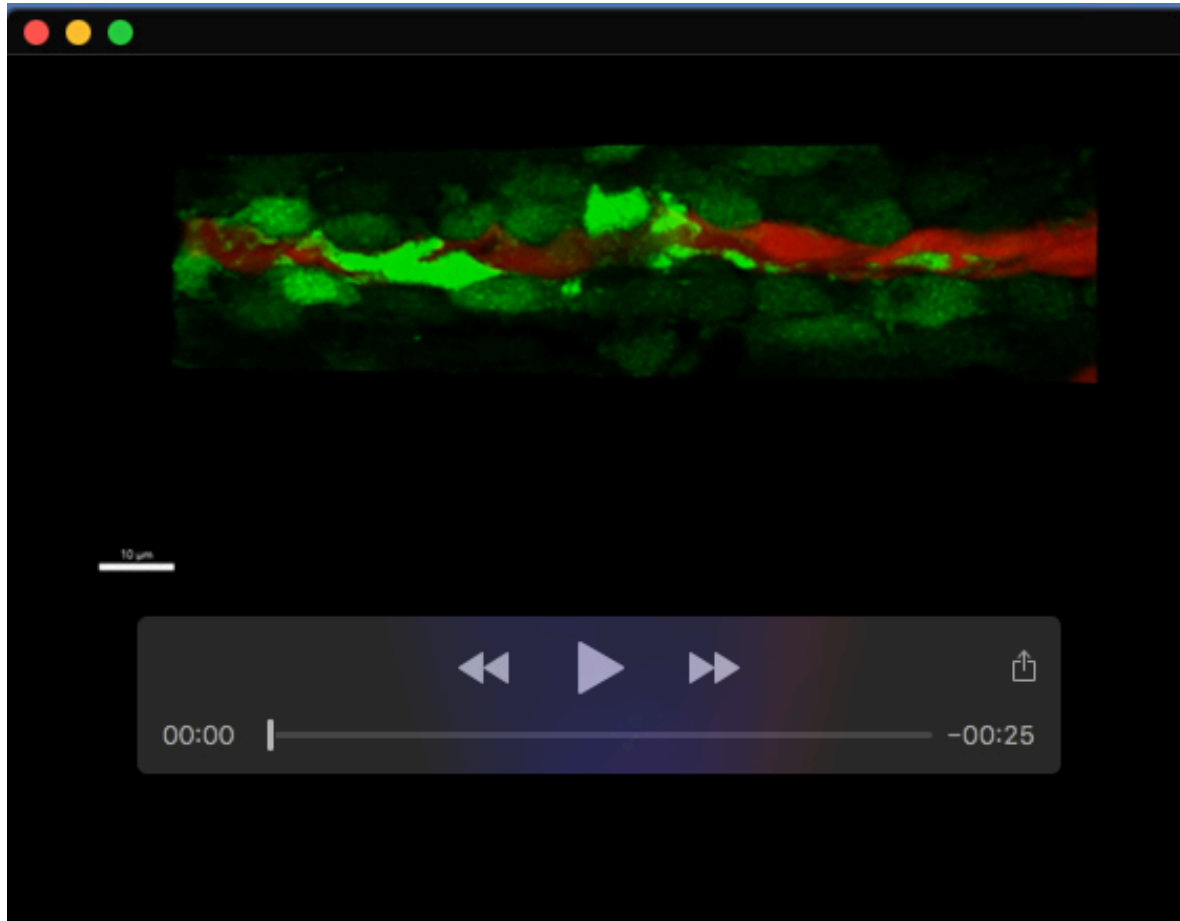
Pdgfrb FP	GCGCTTCAGGCGGGACGAGAA
Pdgfrb RP	CGTCACCAGACATGGGATCGTGC
Tagln FP	GTGGGTCAGCGGTGGGGAGA
Tagln RP	CCTCCCACAGATCCACCGTCT
Myh11a FP	GACATCCAGAAGATGAACCCGCCC
Myh11a RP	GTGAGGAGGGACCTCATGGCGC
Msx1b FP	GACGACAGTGAAGAATAAGCGATAAGG
Msx1b RP	AGAACTCGGCCCGTTTCGGCGAT
Twist1a FP	GGTACATTGACTTCCTCTGTCAGGTC
Twist1a RP	CGCCCTTGTTTTGAGCCGCTCCTT
Pdgfra FP	CTGTGCTTTATGACCCCAAACCTTGGC
Pdgfra RP	CCATTCCAGCAGGAGTCTCACAGG
Col5a1 FP	CCATGTTTCCGGAGGACTTCTCCAT
Col5a1 RP	CTCTGTTTGTGAACGCTGATCGCTAC
Col1a2 FP	GAGGTGGATGCCACCATCAAGTC
Col1a2 RP	GCAGGTCTGGCCAGTAGAGAAGT
Rpl13a FP	TCTGGAGGACTGTAAGAGGTATGC
Rpl13a RP	AGACGCACAATCTTGAGAAGCAG
Acta 2 FP	CATTCGTCACCACTGCTGAAAGAGAAATC
Acta 2 RP	GGCTGGAACAGCGTCTCAGGAC



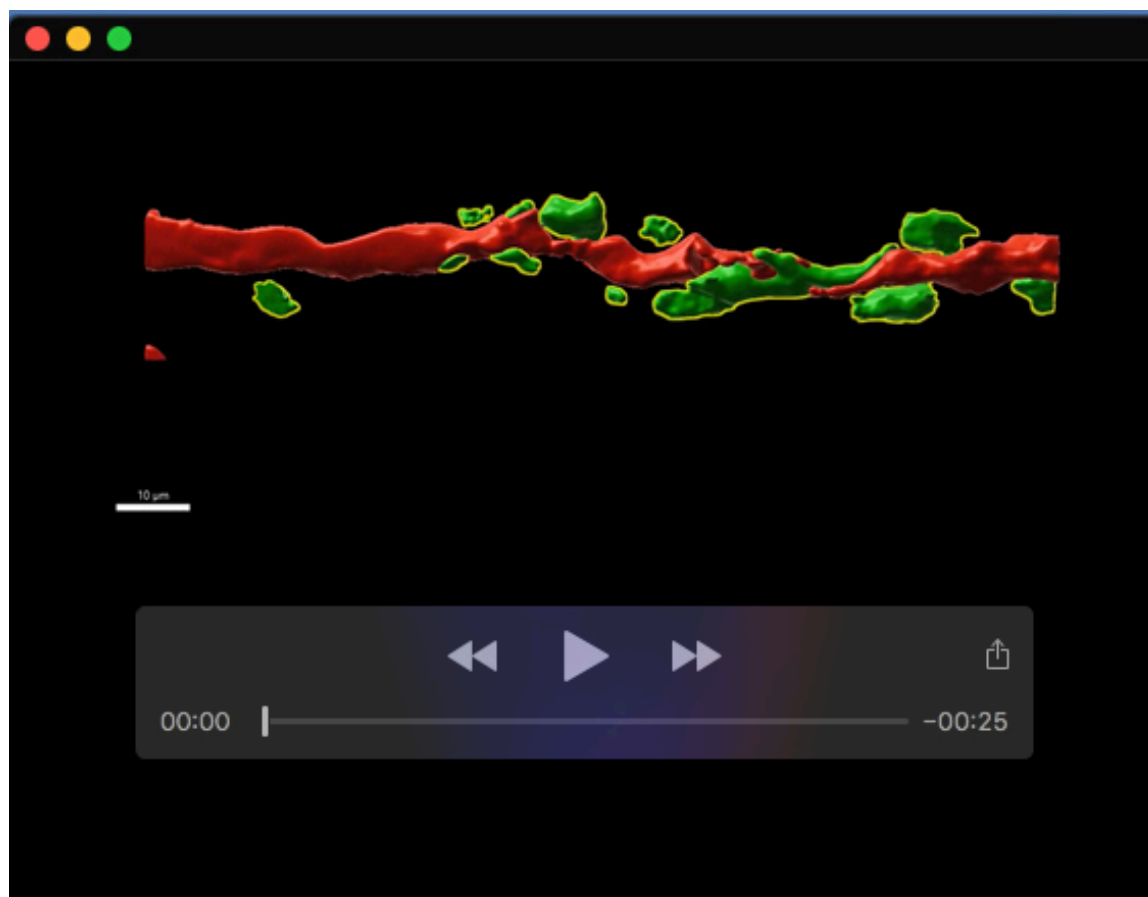
Movie 1. Three-dimensional rendering of fin artery (red) and mural cells (green) in proximal fin regions in a wildtype animal. Transgenic lines used are *TgBAC(pdgfrb:gal4)^{ncv24}*, *Tg(UAS:GFP)^{nkuasgfp1a}*; *Tg(-0.8flt:RFP)^{hu5333}*.



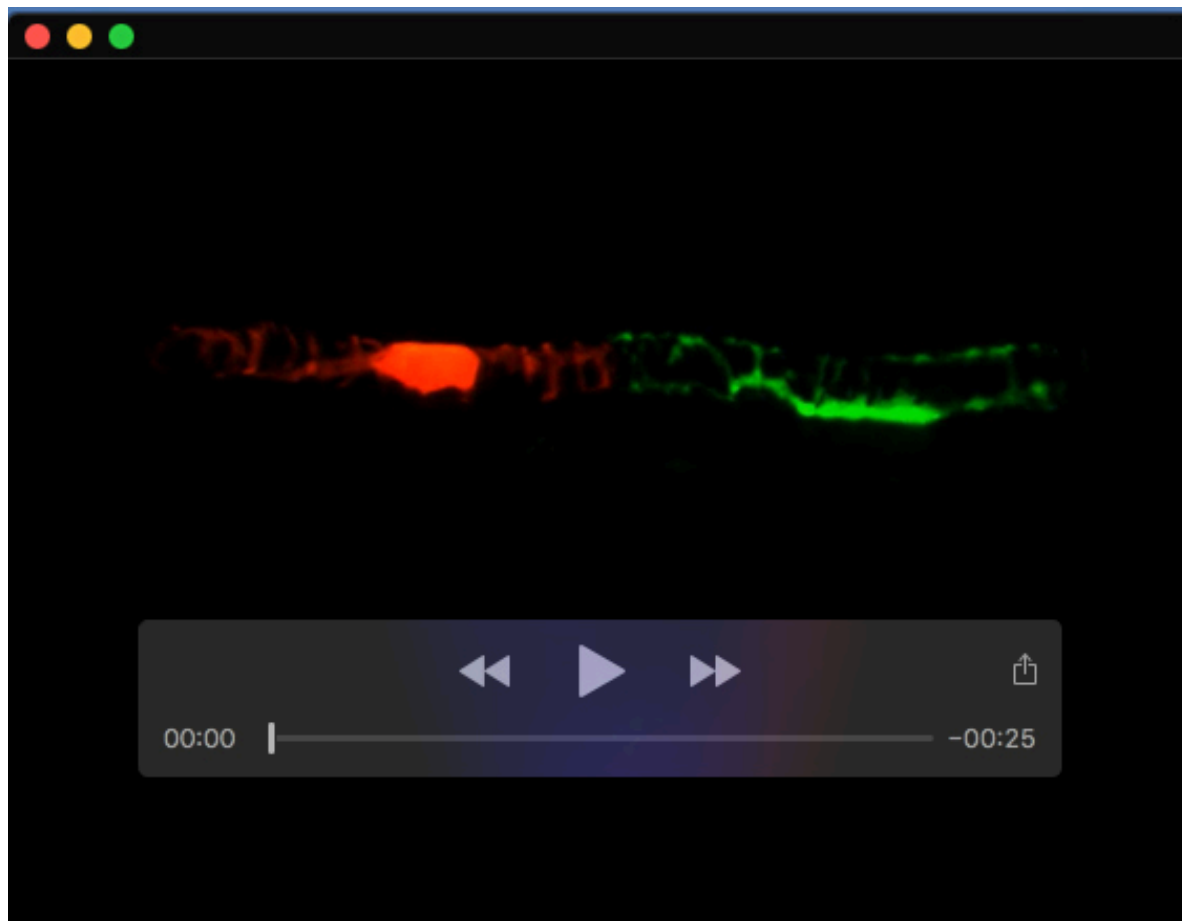
Movie 2. Three-dimensional rendering and registration of fin artery (red) and mural cells (green) in proximal fin regions in a wildtype animal. Transgenic lines used are *TgBAC(pdgfrb:gal4)^{ncv24}*; *Tg(UAS:GFP)^{nkuasgfp1a}*; *Tg(-0.8flt:RFP)^{hu5333}*.



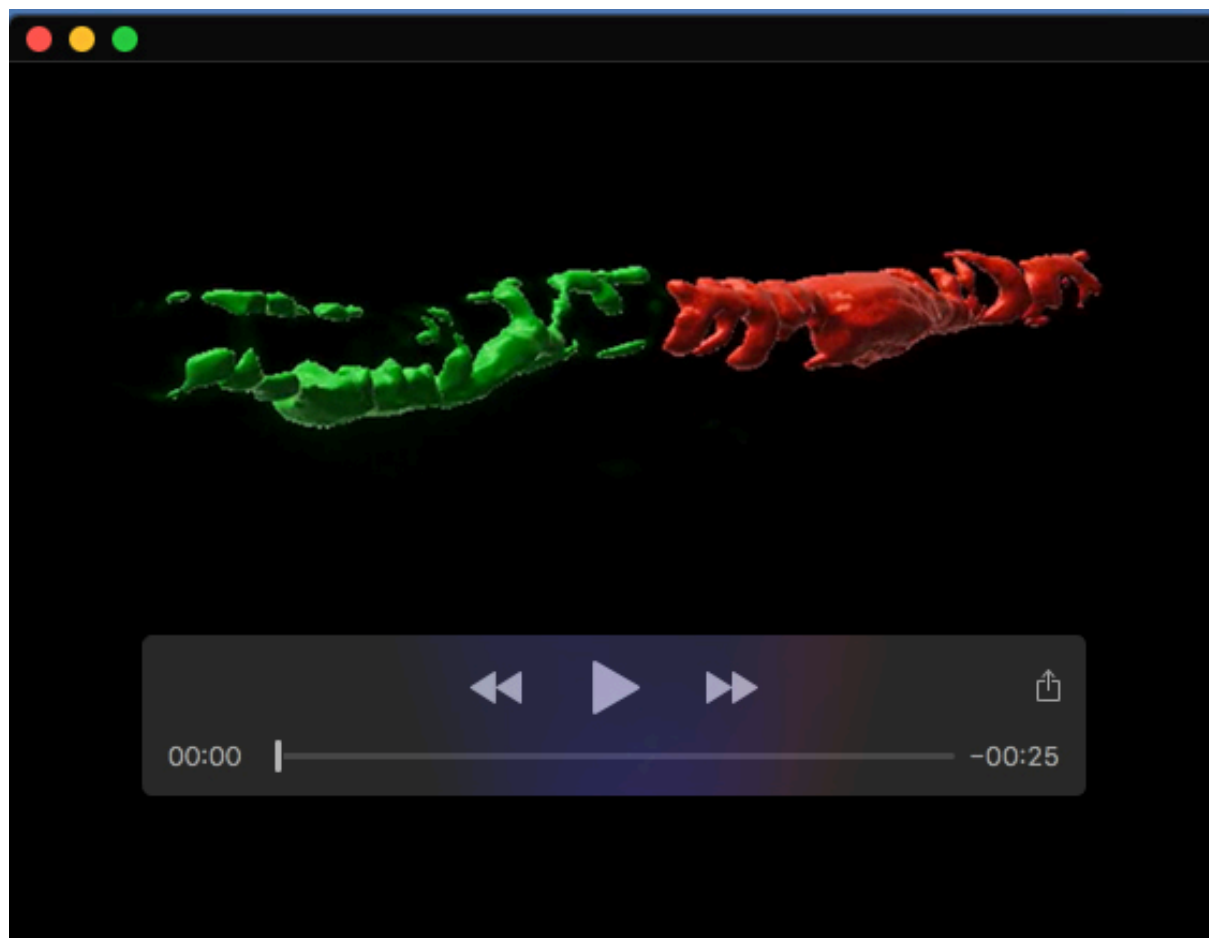
Movie 3. Three-dimensional rendering of fin artery (red) and mural cells (green) in distal fin regions in a wildtype animal. Transgenic lines used are *TgBAC(pdgfrb:gal4)^{ncv24}*; *Tg(UAS:GFP)^{nkuasgfp1a}*; *Tg(-0.8flt:RFP)^{hu5333}*.



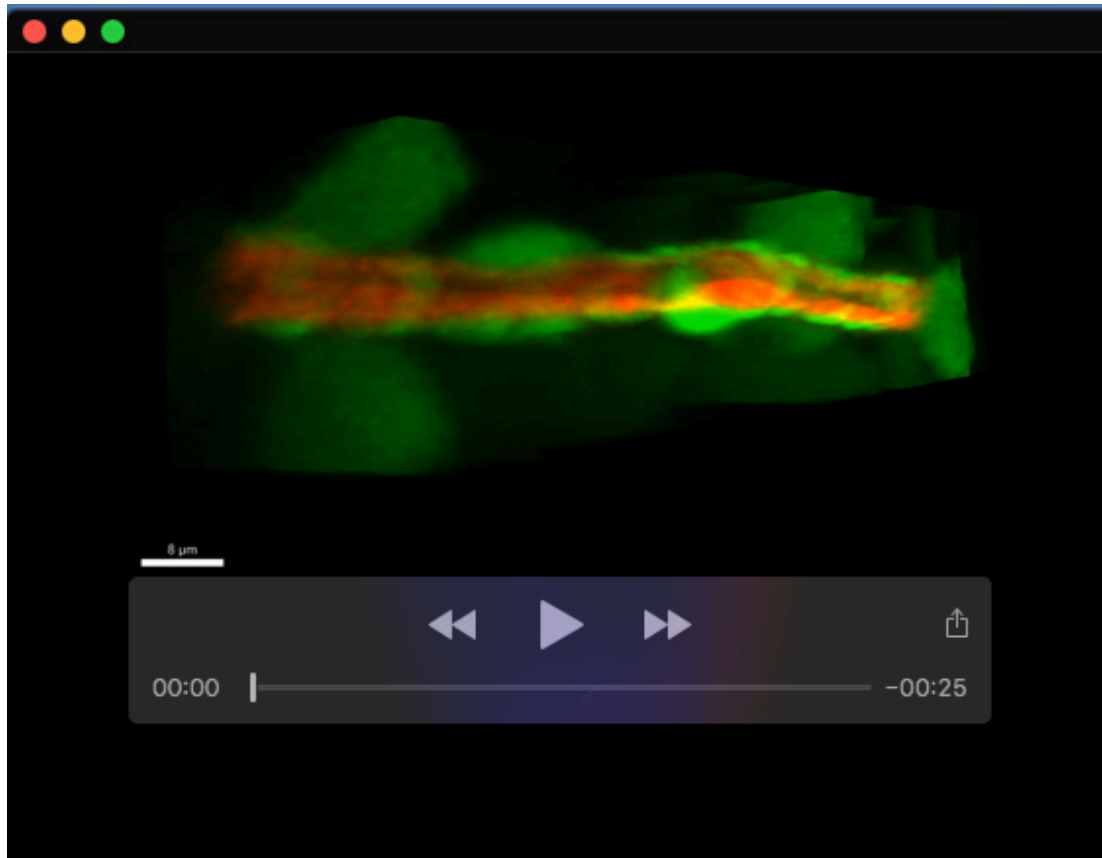
Movie 4. Three-dimensional rendering and registration of fin artery (red) and mural cells (green) in distal fin regions in a wildtype animal. Transgenic lines used are *TgBAC(pdgfrb:gal4)^{ncv24}*; *Tg(UAS:GFP)^{nkuasgfp1a}*; *Tg(-0.8flt:RFP)^{hu5333}*.



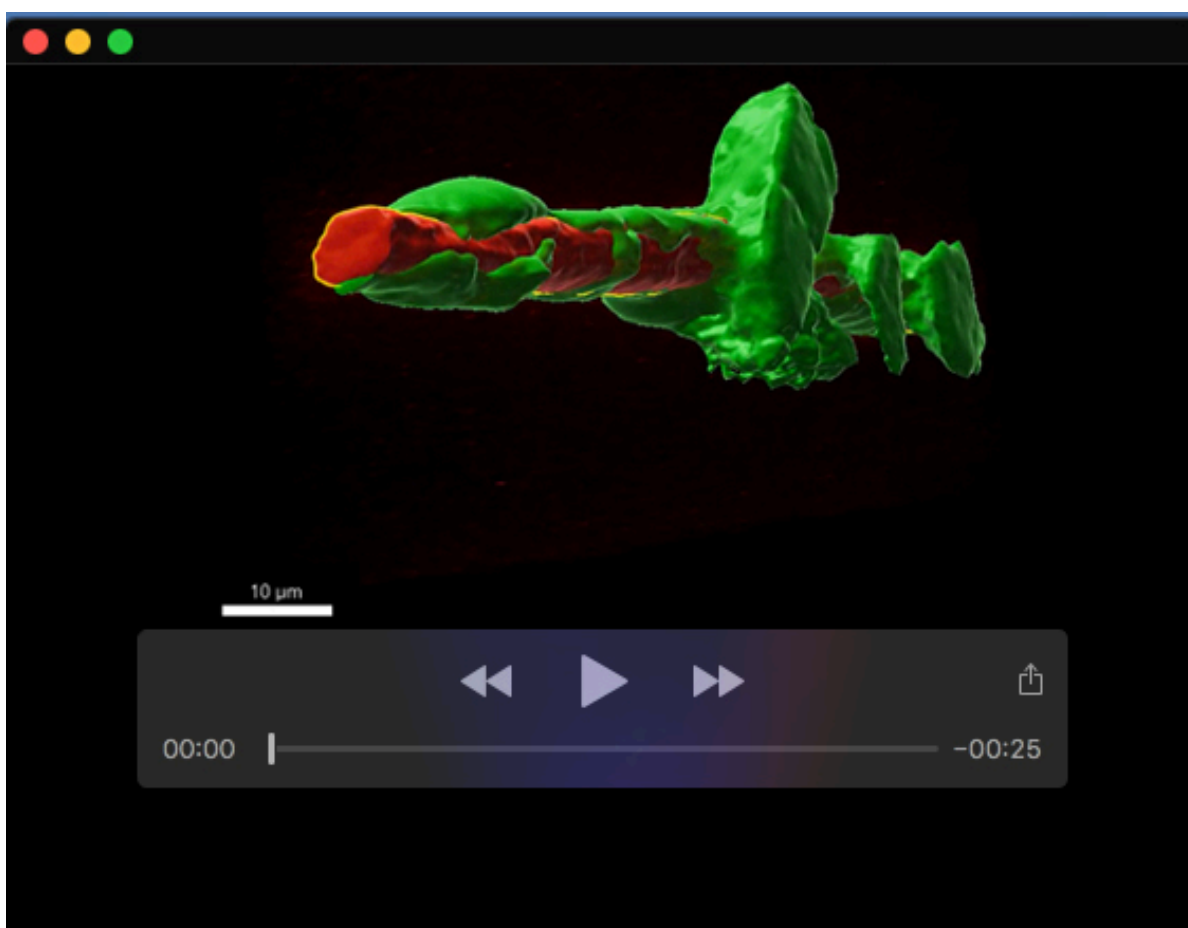
Movie 5. Three-dimensional rendering of individual fin mural cells. Transgenic lines used are *TgBAC(pdgfrb:gal4)^{ncv24}*; *Tg(UAS:KAEDE)^{s1999t}*. One cell was photoconverted prior to imaging (red cell), while the other cell (green) was not photoconverted.



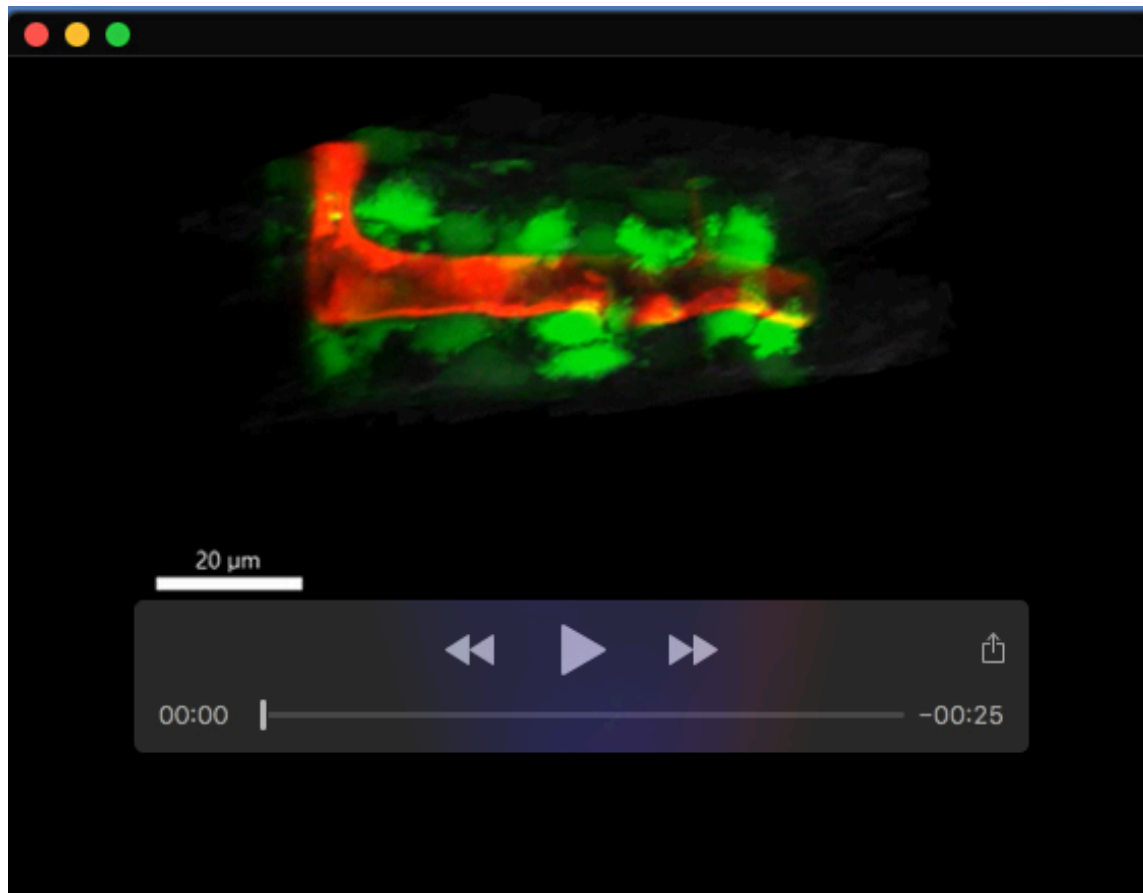
Movie 6. Three-dimensional rendering and registration of individual fin mural cells. Transgenic lines used are *TgBAC(pdgfrb:gal4)^{ncv24}*; *Tg(UAS:KAEDE)^{s1999t}*. One cell was photoconverted prior to imaging (red cell), while the other cell (green) was not photoconverted.



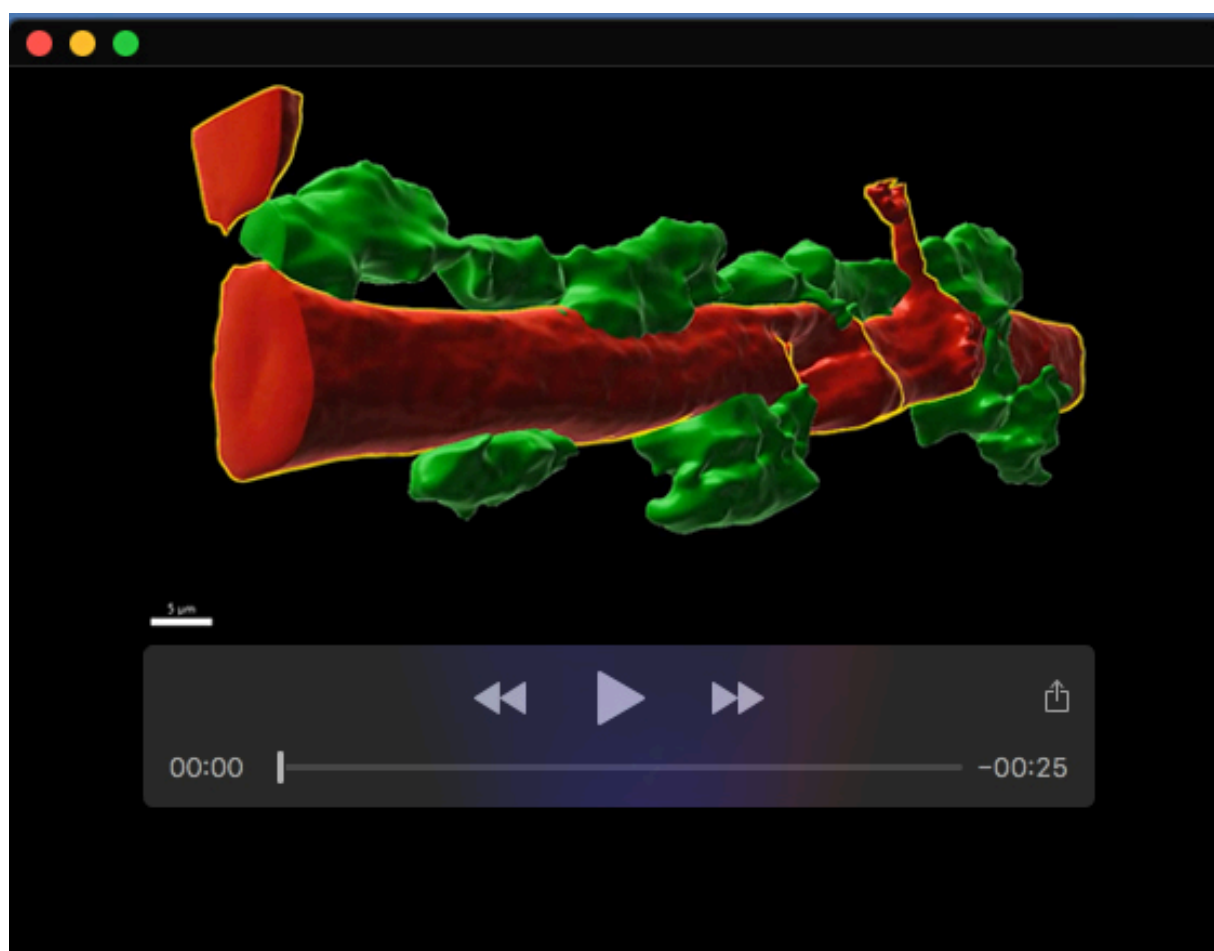
Movie 7. Three-dimensional rendering of fin artery (red) and mural cells (green) in proximal fin regions in a *pdgfrb^{mu158}* animal. Transgenic lines used are *TgBAC(pdgfrb:gal4)^{ncv24}*; *Tg(UAS:GFP)^{nkuasgfp1a}*; *Tg(-0.8ft:RFP)^{hu5333}*.



Movie 8. Three-dimensional rendering and registration of fin artery (red) and mural cells (green) in proximal fin regions in a *pdgfrb^{mu158}* animal. Transgenic lines used are *TgBAC(pdgfrb:gal4)^{ncv24}*; *Tg(UAS:GFP)^{nkuasgfp1a}*; *Tg(-0.8ft:RFP)^{hu5333}*.



Movie 9. Three-dimensional rendering of fin artery (red) and mural cells (green) in distal fin regions in a *pdgfrb*^{mu158} animal. Transgenic lines used are *TgBAC(pdgfrb:gal4)^{hcv24}*, *Tg(UAS:GFP)^{nkuasgfp1a}*, *Tg(-0.8flt:RFP)^{hu5333}*.



Movie 10. Three-dimensional rendering and registration of fin artery (red) and mural cells (green) in distal fin regions in a *pdgfrb*^{mu158} animal. Transgenic lines used are *TgBAC(pdgfrb:gal4)^{hcv24}*, *Tg(UAS:GFP)^{nkuasgfp1a}*, *Tg(-0.8flt:RFP)^{hu5333}*.